

PATENT

Attorney Docket No. 020552-001410US
[Client Ref. No. 1029.1]

Kip3A

864 aa full length sequence

The motor domain is amino acids 348–1174 or base pair 143–1174.

5 GCGGCCGCGAATTGGGACCCGTGAGCAAGCAGCGACGCCAGCGCGGAGAACGACGAAAGGTGT
 10 61 GTGGCTGTGGGACCCGTGAGCAAGCAGCGACGCCAGCGCGGAGAACGACGAAAGGTGT
 15 121 CACCACAGTGATGGCAGTGGAGGA~~G~~AGCACGCTGCAAGTAGTGGTACGGGTGCGGCCCC
 MetAlaValGlu~~Asp~~SerThrLeuGlnValValValArgValArgProPr
 20 181 CACCCCTCGGGAGCTGGACAGTCAGCGCGGCCAGTGGTTCAGGTGGTGGACGAGCGGGT
 oThrProArgGluLeuAspSerGlnArgArgProValValGlnValValAspGluArgVa
 25 241 GCTGGTGTAAACCTGAGGAGCCGATGGAGGGTCCCTGGCCTGAAATGGGTGGCAC
 1LeuValPheAsnProGluGluProAspGlyGlyPheProGlyLeuLysTrpGlyGlyTh
 30 301 CCATGATGGCCCCAAGAAGAAGGGCAAAGACCTGACGTTGTCTTGACCGGGCTTTGG
 rHisAspGlyProLysLysGlyLysAspLeuThrPheValPheAspArgValPheGl
 35 361 CGAGGCGGCCACCCAACAGGACGTGTTCCAGCACACCACGACAGCGTCCGGACAGCTT
 yGluAlaAlaThrGlnGlnAspValPheGlnHisThrThrHisSerValLeuAspSerPh
 40 421 CCTCCAGGGCTACAACGTGCTAGTGTTCGCTACGGGCCACGGGGCTGGAAAGACACA
 eLeuGlnGlyTyrAsnCysSerValPheAlaTyrGlyAlaThrGlyAlaGlyLysThrHi
 45 481 CACCATGCTGGGAAGGGAGGGGACCCCGGCATCATGTACCTGACCACCGTGGAACTGTA
 sThrMetLeuGlyArgGluGlyAspProGlyIleMetTyrLeuThrThrValGluLeuTy
 50 541 CAGGCGCTGGAGGCCGCCAGGAGGAAGCAGGAGAAGCAGTGGAGGTGCTCATCAGCTACCAGGA
 rArgArgLeuGluAlaArgGlnGlnGluLysHisPheGluValLeuIleSerTyrGlnG1

Fig. 1A

Joe Liebeschuetz, Reg. No. 37,505 (650) 326-2400

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5 GGTGTATAATGAAACAGATCCATGACCTCCTGGAGCCAAAGGGGCCCTTGCCATCCGCGA
 601 -----+-----+-----+-----+-----+-----+-----+-----+
 uValTyrAsnGlnIleHisAspLeuLeuGluProLysGlyProLeuAlaIleArgGl
 661
 10 GGACCCCGACAAGGGGGTGGTGGTCAAGGACTTCTTCACCAGCCAGCCTCAGCCGA
 661 -----+-----+-----+-----+-----+-----+-----+
 uAspProAspLysGlyValValValGlnGlyLeuSerPheHisGlnProAlaSerAlaGl
 721
 15 GCAGCTGCTGGAGATACTGACCAGGGGAAACCGTAACCGCACGCAGCACCCCCTGATGC
 721 -----+-----+-----+-----+-----+-----+-----+
 uGlnLeuLeuGluIleLeuThrArgGlyAsnArgAsnArgThrGlnHisProThrAspAl
 781
 20 CAACCGCAGTTCCCTCCCGCTCCCATGCCATCTTCAGATCTTGTGAAGCAGCAGGACCG
 781 -----+-----+-----+-----+-----+-----+-----+
 aAsnAlaThrSerSerArgSerHisAlaIlePheGlnIlePheValLysGlnGlnAspAr
 841
 25 GGTTCCAGGACTGACCCAGGCTGTCCAGGTGGCCAAGATGAGCCTGATTGACCTGGCTGG
 841 -----+-----+-----+-----+-----+-----+-----+
 gValProGlyLeuThrGlnAlaValGlnValAlaLysMetSerLeuIleAspLeuAlaGl
 901
 30 CTCAGAGCGGGCATCCAGCACCCATGCGAAGGGGGAGCGGGCTGCGGGAGGGGCCAACAT
 901 -----+-----+-----+-----+-----+-----+-----+
 ySerGluArgAlaSerSerThrHisAlaLysGlyGluArgLeuArgGluGlyAlaAsnIl
 961
 35 CAACCGCTCTGCTGGCGCTCATCACGTCTCAATGCCTGGCCATGCAAAGGGCCG
 961 -----+-----+-----+-----+-----+-----+-----+
 eAsnArgSerLeuLeuAlaLeuIleAsnValLeuAsnAlaLeuAlaAspAlaLysGlyAr
 1021
 40 CAAGACCCATGTGCCCTACCGGGACAGCAAACGTGACCCGCTGCTCAAAGACTCCCTCGG
 1021 -----+-----+-----+-----+-----+-----+-----+
 gLysThrHisValProTyrArgAspSerLysLeuThrArgLeuLeuLysAspSerLeuGl
 1081
 45 GGGCAACTGCCGCACAGTGATGATCGCTGCCATCAGCCCCTCCAGCCTGACCTACGAGGA
 1081 -----+-----+-----+-----+-----+-----+-----+
 yGlyAsnCysArgThrValMetIleAlaAlaIleSerProSerSerLeuThrTyrGluAs
 1141
 50 CACGTACAACACCCCTCAAATATGCCGACCGG~~GC~~CAAGGAGATCAGGCTCTCGCTGAAGAG
 1141 -----+-----+-----+-----+-----+-----+-----+
 pThrTyrAsnThrLeuLysTyrAlaAspArgAlaLysGluIleArgLeuSerLeuLysSe
 1201
 55 CAATGTGACCAGCCTGGACTGTACATCAGCCAGTATGCTACCATCTGCCAACAGCTCCA
 1201 -----+-----+-----+-----+-----+-----+-----+
 rAsnValThrSerLeuAspCysHisIleSerGlnTyrAlaThrIleCysGlnGlnLeuGl

Fig. 1B

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1261 GGCTGAGGTAGCCGCTCTGAGGAAGAACGCTCAAGTGTATGAGGGGGAGGCCAGCCCCC 1320
5 nAlaGluValAlaAlaLeuArgLysLysLeuGlnValTyrGluGlyGlyGlyGlnProPr
1321 ACCACAGGACCTCCCAGGATCTCCAAGTCGGGACCACCAGAACACCTCCAGCTC 1380
10 oProGlnAspLeuProGlySerProLysSerGlyProProProGluHisLeuProSerSe
1381 CCCCTGCCACCCACCCCTCCCAGCCAGCCCTGCACCCCAGAGCTCCCTGCAGGGCCTAG 1440
15 rProLeuProProHisProProSerGlnProCysThrProGluLeuProAlaGlyProAr
1441 AGCCCTTCAAGAGGGAGAGTCTGGGGATGGAGGCCAGGTGGAGAGGCCATGGAAGGGAA 1500
20 gAlaLeuGlnGluGluSerLeuGlyMetGluAlaGlnValGluArgAlaMetGluGlyAs
1501 CTCTTCAGACCAGGAGCACTCCCCAGAGGATGAGGATGAAGGCCAGCTGAGGAGGTTCC 1560
25 nSerSerAspGlnGluGlnSerProGluAspGluAspGluGlyProAlaGluGluValPr
1561 AACCCAGATGCCAGAGCAGAACCCACACATGCACTGCCAGAGTCCCCTGCCCTGACCC 1620
30 oThrGlnMetProGluGlnAsnProThrHisAlaLeuProGluSerProArgLeuThrLe
1621 GCAGCCCAAGCCAGTCGTGGCCACTTCTCAGCACGGAACTGGATGGGACCGTTCTAA 1680
35 uGlnProLysProValValGlyHisPheSerAlaArgGluLeuAspGlyAspArgSerLy
1681 GCAGTTGGCCCTAAAGGTGCTGTGCGTTGCCAGCGGCAGTACTCCCTGCTCCAAGCAGC 1740
40 sGlnLeuAlaLeuLysValLeuCysValAlaGlnArgGlnTyrSerLeuLeuGlnAlaAl
1741 CAACCTCCTGACGCCGACATGATCACAGAGTTGAGACCCTACAGCAGCTGGTGCAAGA 1800
45 aAsnLeuLeuThrProAspMetIleThrGluPheGluThrLeuGlnGlnLeuValGlnG
1801 GGAAAAAAATTGAGCCCTGGGGCAGAGGCCTTGAGGACTTCAGGCCTGCCAGGGGGCACC 1860
50 uGluLysIleGluProGlyAlaGluAlaLeuArgThrSerGlyLeuAlaArgGlyAlaPr
1861 TCTGGCTCAGGAGCTGTGTTCAAGTCATCCCTGTGCCGTCTCCTCTGCCAGAGCC 1920
55 oLeuAlaGlnGluLeuCysSerGluSerIleProValProSerProLeuCysProGluPr

Fig. 1C

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1921 TCCAGGATACACTGGCCCTGTGACCCGGACTATGGCGAGGCGACTGAGTGGCCCCCTGCA 1980
5 oProGlyTyrThrGlyProValThrArgThrMetAlaArgArgLeuSerGlyProLeuHi
1981 CACCTGGGAATCCCGCCTGGACCCAAC TGACCCAGCCCAGGGTCCCGATGGCCCAT 2040
10 sThrLeuGlyIleProProGlyProAsnCysThrProAlaGlnGlySerArgTrpProMe
2041 GGAGAAGAAGAGGAGGGAGACCAAGCGCCTTGGAGGCAGACAGTCCCATGGCCTCAAAGCG 2100
15 tGluLysLysArgArgProSerAlaLeuGluAlaAspSerProMetAlaSerLysAr
2101 GGGCACCAAGGCCAGCGCAGTCCTCCTGCCCTGCCCTAAGGAGAGGGTCTGCCTGA 2160
20 gGlyThrLysArgGlnArgGlnSerPheLeuProCysLeuArgArgGlySerLeuProAs
2161 CACCAACCTTCACAGGGGCCAGCACCCCCAAAGGAGAAAGGGCCTCCTCCCCCTGCCA 2220
25 pThrGlnProSerGlnGlyProSerThrProLysGlyGluArgAlaSerSerProCysHi
2221 TTCCCCCTCGCGTTGCCAGCCACAGTCATCAAAAGCCGGGTGCCCTGGGCCCTCCGC 2280
30 sSerProArgValCysProAlaThrValIleLysSerArgValProLeuGlyProSerAl
2281 CATGCAGAACTGCTCCACCCGCTGGCTTGCCCACTCGAGACCTCAATGCCACCTTG 2340
35 aMetGlnAsnCysSerThrProLeuAlaLeuProThrArgAspLeuAsnAlaThrPheAs
2341 TCTCTCTGAGGAGCCTCCCTCAAAGCCCAGTTCCATGAATGCATTGGCTGGACAAAAT 2400
40 pLeuSerGluGluProProSerLysProSerPheHisGluCysIleGlyTrpAspLysI
2401 ACCCCAGGAGCTGAGCAGGCTGGACCAGCCCTTCATCCCCAGGGCACCTGTGCCCTGTT 2460
45 eProGlnGluLeuSerArgLeuAspGlnProPheIleProArgAlaProValProLeuPh
2461 CACCATGAAGGGCCCAAGCCAACATCTCCCTCCCTGGACCTCTGCCCTGCAAGAAGAA 2520
50 eThrMetLysGlyProLysProThrSerSerLeuProGlyThrSerAlaCysLysLy
2521 GCGCGTTGCGAGTTCCCTCAGTCTCCATGGCCGCAGCCGCATGCCCGCCTCCGAGCAG 2580
55 sArgValAlaSerSerSerValSerHisGlyArgSerArgIleAlaArgLeuProSerSe

Fig. 1D

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2581	CACTTTGAAGAGGCCAGCTGGCCCTTGTACTCCCAGAGCTGCCCTGAGTCCCCTGTG rThrLeuLysArgProAlaGlyProLeuValLeuProGluLeuProLeuSerProLeuCy	2640
5		
2641	CCCTAGCAACCGGAGGAATGAAAGGACCTCATCAGGGTGGGAGAGCGCTCTCAGCAGG sProSerAsnArgArgAsnGlyLysAspLeuIleArgValGlyArgAlaLeuSerAlaGly	2700
10		
2701	GAACGGCGTCACCAAGGTGCTTGACCGCCAGAATGCTTGACCAAGGTGCTTAAC yAsnGlyValThrLysValSer	2760
15		
2761	CTACCGGCCCTTGCTGGATACCCCTCTGGACCTGTAGCCACCTGCACCAGGAGCTGG	2820
20		
2821	ACCTGCCTTCCTTACCTGGAGCAATTAGTGCCAACACACACCTTGCTGTATTAACATCCC	2880
25		
2881	TCCCCAGACATCCATCCTGCTACTCACCCCTCTGTTAATCTCCTGTTACACTCAGCTTCTT	2940
30		
2941	GGCATGTACATATTCAATTGTGAGTGTTAATGTGCTGCTGTTTTGTTTTGGTGGTT	3000
35		
3001	TTTGTTTTGTGTTTTGTTTGAGATGGAGTCTTACTCTGTCGCCAGGCTGGAGTG	3060
40		
3061	CAGTGGTACGATCTGGCTACTGCAACCTCCGCCTGGTTCAAGTAATTCTCCTGC	3120
45		
3121	CTCAGCTTCCAAGTAGCTGGATTACAGGCACCCATCACCACACCCAGCTAATTTCGT	3180
50		
3181	CTTTTAATAGAGAGGGGTTTTCCATGTTGCCAGGCTGGCTTGAACTCCTGACCTC	3240
55		
3241	AGGTGATCCGCCTGCCTCAGCTCCAAAGTGCTGAGATTACAGGCATGAGCTACCACGC	3300
55		
3301	CTGGCCCGTGTGCTGTTAAAGGTGCTGCCATGTTCCCCATTTTTTTTGAG	3360

Fig. 1E

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Fig. 1F.

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DNA sequence of HsKIP3A, motor only

1 GACAGCACGC TGCAAGTAGT GGTACGGGTG CGGCCCCCA CCCCTCGGA GCTGGACAGT
61 CAGCGCGGC CAGTGGTTCA GGTGGTGGAC GAGCGGGTGC TGGTGTAA CCTTGAGGAG
5 121 CCCGATGGAG GGTCCCTGG CCTGAAATGG GGTGGCACCC ATGATGGCCC CAAGAAGAAG
181 GGCAAAGACC TGACGTTTGT CTTTGACCGG GTCTTTGGCG AGGCGGCCAC CCAACAGGAC
241 GTGTTCCAGC ACACCCACGCA CAGCGTCCTG GACAGCTTC TCCAGGGCTA CAACTGCTCA
301 GTGTTGCCT ACGGGGCAC CGGGGCTGGG AAGACACACA CCATGCTGGG AAGGGAGGGG
361 GACCCCGGCA TCATGTACCT GACCACCGTG GAACTGTACA GGCGCTGGA GGCCCGCCAG
421 CAGGAGAAGC ACTTCGAGGT GCTCATCAGC TACCAGGAGG TGTATAATGA ACAGATCCAT
10 481 GACCTCCTGG AGCCCAAGGG GCCCCTTGCC ATCCGCGAGG ACCCCGACAA GGGGGTGGT
541 GTGCAAGGAC TTTCTTTCCA CCAGCCAGCC TCAGCCGAGC AGCTGCTGGA GATACTGACC
601 AGGGGAACC GTAACCGCAC GCAGCACCCC ACTGATGCCA ACGCGACTTC CTCCCGCTCC
661 CATGCCATCT TCCAGATCTT TGTGAAGCAG CAGGACCGGG TTCCAGGACT GACCCAGGCT
721 GTCCAGGTGG CCAAGATGAG CCTGATTGAC CTGGCTGGCT CAGAGCGGGC ATCCAGCACC
15 781 CATGCGAAGG GGGAGCGGT GCGGGAGGGG GCCAACATCA ACCGCTCTCT GCTGGCGCTC
841 ATCAACGTCC TCAATGCCTT GGCGATGCA AAGGGCCGCA AGACCCATGT GCCCTACCGG
901 GACAGCAAAC TGACCCGCCT GCTCAAAGAC TCCCTCGGGG GCAACTGCCG CACAGTGTG
961 ATCGCTGCCA TCAGCCCCCTC CAGCCTGACC TACGAGGACA CGTACAAACAC CCTC

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Fig. 2

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Protein sequence of HsKIP3A, motor only

5 1 DSTLQVVVRV RPPTPRELDS QRRPVVQVVD ERVLVFNPEE PDGGFPGLKW GGTHDGPKKK
61 61 GKDLTFVFDR VFGEAAATQD VFQHTTHSVL DSFLQGYNCS VFAYGATGAG KTHTMLGREG
121 121 DPGIMYLTIV ELYRRLEARQ QEKHFEVLIS YQEYVYNEQIH DLLEPKGPLA IREDPDKGVV
181 181 VQGLSFHQPA SAEQLLEILIT RGNRNRTOQHP TDANATSSRS HAIFQIFVKQ QDRVPGLTQA
241 241 VQVAKMSLID LAGSERASST HAKGERLREG ANINRSLLAL INVNLNALADA KGRKTHVPYR
301 301 DSKLTRLKD SLGGNCRTVM IAAISPSSLT YEDTYNTL

10 Fig. 3

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Figure 4:

MAVEDSTLQVVVRVRPPTPRELDSQRRPVVQVVDERVLVFNPEEPDGGFPGLKWGGT
HDGPKKKGKDLTFVFDVFGEAATQQDVFQHTTHSVLDSFLQGYNCSVFAYGATGAG
KTHTMLGREGDPGIMYLTTVELYRRLEARQQEKHFELISYQEVYNEQIHDLLEPKG
5 PLAIREDPDKVQQVQGLSFHQPASAEQILLEILTRGNRNRTQHPTDANATSSRSHAIF
QIFVKQQDRVPGLTQAVQVAKMSLIDLGSERASSTHAKGERLREGANINRSLLALI
NVLNALADAKGRKTHVPYRDSLTRLLKDSLGGNCRTVMIAAISPSSLTYEDTYNTL
KYADRAKEIRLKGNNSKLEGKPIPNPLLGLDSTRTGHHHHHH

10 Grayed sequence is derived from the vector (pCRT7/CT, Invitrogen) and contains a V5 epitope and polyhistidine tag.

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Figure 5: Nucleotide sequence of the Kip3a fragment used in the ATPase assay

ATGGCAGTGGAGGACAGCACGCTGCAAGTAGTGGTACGGGTGCGGCCCCACCCCT
CGGGAGCTGGACAGTCAGCGCGGCCAGTGGTCAGGTGGACGAGCGGGTGCTG
5 GTGTTAACCTGAGGAGCCGATGGAGGGTCCCTGGCCTGAAATGGGTGGCACC
CATGATGGCCCCAAGAAGAAGGGCAAAGACCTGACGTTGTCTTGACCAGGGTCTT
GGCAGGGCGGCCACCCAAACAGGACGTGTTCCAGCACACCACGCACAGCGTCTGGAC
AGCTTCCTCCAGGGCTACAAC TGCTCAGTGGTGCCTACGGGGCCACCGGGGCTGGG
10 AAGACACACACCATGCTGGGAAGGGAGGGGACCCGGCATCATGTACCTGACCACC
GTGGAAC TGACAGGCGCTGGAGGCCAGCAGGAGAACGACTTCGAGGTGCTC
ATCAGCTACCAGGAGGTGTATAATGAACAGATCCATGACCTCCTGGAGGCCAAGGGG
CCCCTGCCATCCCGAGGACCCGACAAGGGGGTGGTGGTGCAGGACTTTCTTC
CACCAAGCCAGCCTCAGCCGAGCAGCTGCTGGAGATACTGACCAGGGGAACCGTAAC
CGCACGCAGCACCCACTGATGCCAACCGACTTCCTCCGCTCCATGCCATCTTC
15 CAGATCTTGTGAAGCAGCAGGACCGGGTTCCAGGACTGACCCAGGCTGTCCAGGTG
GCCAAGATGAGCCTGATTGACCTGGCTGGCTCAGAGCGGGCATCCAGCACCCATGCG
AAGGGGGAGCGGCTGGGGAGGGGCAACATCAACCGCTCTGCTGGCGCTCATC
AACGTCCCTCAATGCCCTGGCCGATGCAAAGGGCGCAAGACCCATGTGCCCTACCGG
GACAGCAA ACTGACCCGCTGCTCAAAGACTCCCTGGGGCAACTGCCGACAGTG
20 ATGATCGCTGCCATCAGCCCTCCAGCCTGACCTACGAGGACACGTACAACACCCCTC
AAATATGCCGACCAGGGCAAGGAGATCAGGCTCAAGGGCAATTGAAAGCTGAAGGT
AAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCTACGGTACCGGTATCATCAC
CATCACCATG

25 Grayed sequence is derived from the vector (pCRT7/CT, Invitrogen) and contains a V5 epitope and polyhistidine tag.

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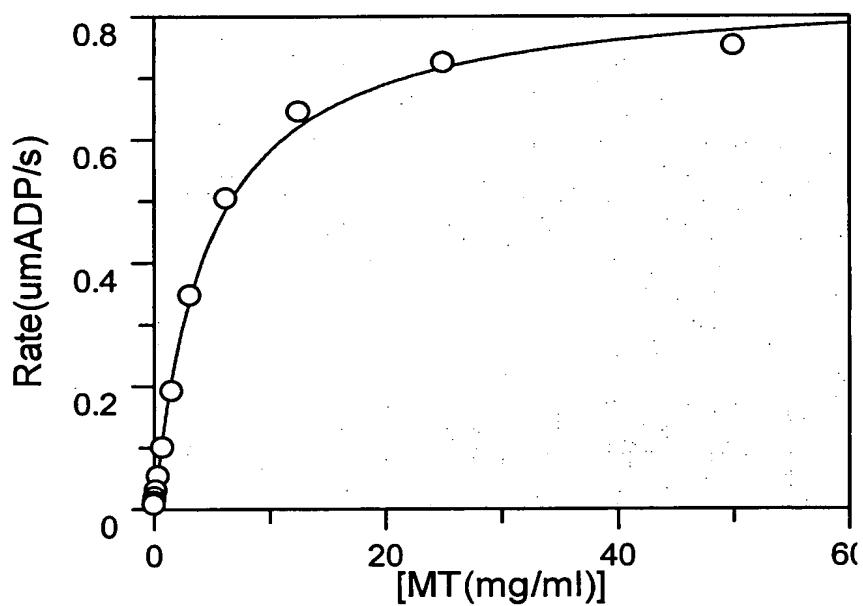
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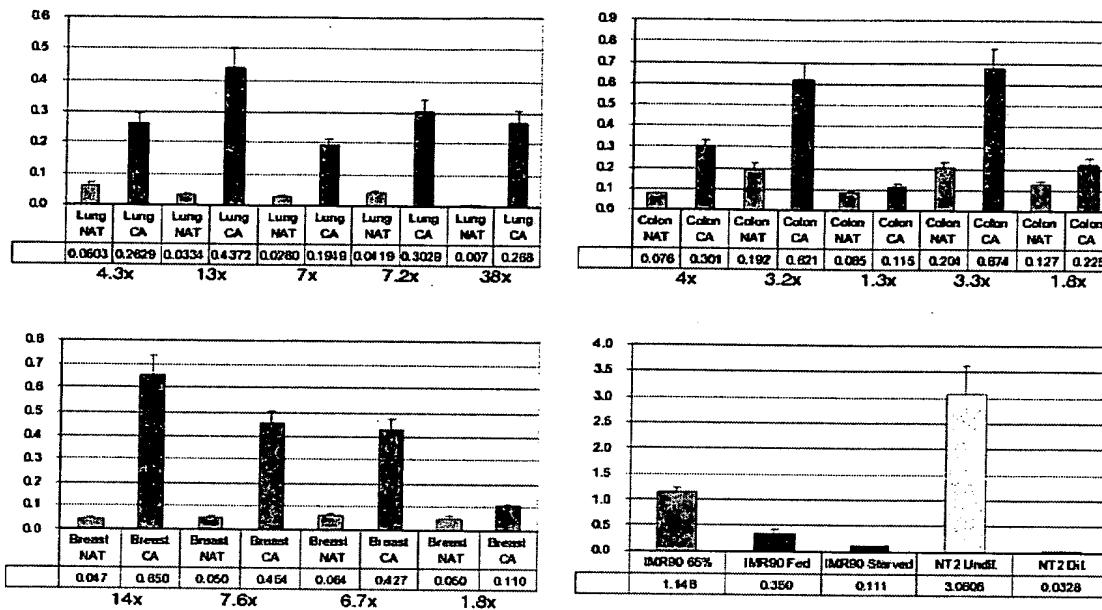
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Fig. 6



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5 Fig. 7

10 PA 3150458 v1

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